In re application of:	Chau, Vincent		
Application No.:	10/681690	Group No.:	1652
Filed:	October 8, 2003	Examiner:	Fronda, Christian, L.
For:	NEDD-8 CONJUGATING ENZYME 1 AND METHODS OF USE		

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

DECLARATION UNDER 37 C.F.R. § 1.131

Sir:

I, Tatiana Gladysheva, hereby declare and state:

1. I am a biochemist who was performing studies at Proscript, Inc. (which later became part of Millennium Pharmaceuticals, Inc.) under the direction of the inventor of the subject matter described and claimed in the above-identified application.

2. I performed the PCR and sequencing studies to identify and characterize the NEDD-8 conjugating enzyme 1 in the United States before August 1, 1998.

3. Evidence is provided by the following:

a) Prior to August 1, 1998, I had completed the sequencing of the overlapping clones which led to the full length NEDD-8 conjugating enzyme 1 (NCE1). Exhibit A is a copy of my notebook pages evidencing studies wherein I performed PCR of cDNA from a human leukocyte library and sequenced the products. The sequences detailed on pages 126-127 are sequences of the PCR products which cover the reverse complement of NCE1. In order, they cover the nucleotides

---

CERTIFICATION UNDER 37 C.F.R. SECTIONS 1.8(a) and 1.10*

I hereby certify that, on the date shown below, this correspondence is being:

MAILING

☑ deposited with the United States Postal Service in an envelope addressed to the Commissioner for Patents, P.O. Box 1450, Alexandria, VA 22313-1450.

37 C.F.R. SECTION 1.8(a)

☑ with sufficient postage as first class mail.

37 C.F.R. SECTION 1.10*

☐ as "Express Mail Post Office to Addressee" Mailing Label No.

TRANSMISSION

☐ transmitted by facsimile to the Patent and Trademark Office.

Signature

Sean Hunziker/Beverly Sotiropoulos

Date: _____

(type or print name of person certifying)

***WARNING:** *Each paper or fee filed by "Express Mail" must have the number of the "Express Mail" mailing label placed thereon prior to mailing. 37 C.F.R. section 1.10(b). "Since the filing of correspondence under section 1.10 without the Express Mail mailing label thereon is an oversight that can be avoided by the exercise of reasonable care, requests for waiver of this requirement will not be granted on petition." Notice of Oct. 24, 1996, 60 Fed. Reg. 56,439, at 56,442.*

363-108, 445-232, 116-1 (with some 5′ untranslated nucleotides), 198-111, 553-360 and 553-500 (with some 3′ untranslated nucleotides). The match of these sequences to the NCE1 nucleotide sequence can be followed by comparing the sequences on my notebook pages to the lower nucleic acid sequence line of Figure 2 of the application. The notebook pages bear the dates on which I performed the studies and the date on which my notebook was witnessed (by ESL). In the original notebook, these dates are prior to August 1, 1998. In accordance with accepted practice, the dates on the copies of the notebook pages have been masked (M.P.E.P. § 715.07).

b) Exhibit B is a copy of an electronic printout of the complete coding and noncoding nucleic acid sequences, matched to the amino acid sequence of NCE1 from Proscript's sequence database. The upper nucleic acid strand on this printout is SEQ ID NO:3 of the application and the amino acid sequence on the printout is SEQ ID NO:4 of the application. These sequences were used for Figure 2 of the application. The printout bears an automatically stamped date of printing and the title, "ubc12." The original printout bears a date prior to August 1, 1998. In accordance with accepted practice, the date on the copy of the electronic printout have been masked (M.P.E.P. § 715.07).

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Gladysheva — 02. 11. 2006

Tatiana Gladysheva — Date

NOTEBOOK NO. 2 NIH

ISSUED TO Tatiana Gladyshtva

ON _____ **19**_

DEPARTMENT Biochemistry

RETURNED _____ **19**_

—SCIENTIFIC NOTEBOOK CO.—
2831 LAWRENCE AVE.
P.O. BOX 238
STEVENSVILLE, MI 49127
616-429-8285

(81)

PCR out the UBCH12 (cDNA human leukocytes) cont. 2

1st run: total volume 100µl

tubes	template	ddH2O	10X-PCR buffer	dNTP 10mM	primers (50pmol/µl) UBCH12FP / UBCH12RP	polymerase
1) (1µl 1:10 dilut.)	0.1µl	7~~6~~.9µl	10µl	10µl	1µl + 1µl	1µl
2)	0.5µl	7~~6~~.5µl	10µl	10µl	1µl + 1µl	1µl

Run the cycles (30) ~ 2.5h (1234 - program):

95°C 30"
55°C 30"
72°C 1'
} 30 times → 4°C forever

2nd run:

tubes	template	ddH2O	10X-PCR buffer	dNTP	primers UBCH12(EcoRI/NdeI)FP; UBCH12(HindIII) RP
I: 1-2	2µl	154µl	20µl	20µl	2µl
II: 3-4	2µl	154µl	20µl	20µl	2µl

Tubes I and II were divided into 1 and 2 (I), and 3 and 4 (II): 99µl in each tube

Polymerase 1µl were added in each of 4 tubes to start the reactions; 100µl is the total volume

Run 30 cycles, the same program (1,2,3,4)

(126)
E52

UBC H12 Sequence

RP1: long

tggcttccagtcctctctgaggatgttgaggcagac
gttgccctcgaggtcaatgttggggtgatagaccattg
tctcacacttcaccttggggggatcatgcgggtaaccc
tggcccaccttaaacctgaacacaaacttcccactcttgt
agaagccctcatcaggacagatgccacagttgaagttga
ggaggtcgtctggatctgagaacgatatcacacgtctt
gggcaggttcagctcgttatgtcc

ggacagatgaccagcttgaagtt

RP1 sh

ggacacacacctctagttcagtgggtcctcg
ttgggctccaagaagaatcatgcaggccatgaa
ttatgagagttatcgtgaggactggctccagtcc
tctctgaggatgttgaggcagacgttgccctcgagg
tcaatgttggggtgatagaccattatctcacac
ttcaccttggggggatcatgcgggtaaccctgg
cccaccttaaacctgaa

RP2 long

ttatgtccttctggatccgcagctgcgccgccgacgac
ttcttgctgctgcccttggtgccgccagccgaccctcctcc
tccttcttctgctgcttcagccgagaacagcttgatc
atatgtatatctccttcttaaagttaaacaaaattattct
agagggaaaccgttgtggtctccctatcgtgagct
gtattaattcgaagtctatcagaagttcgaat

Not
tcttgctg
ctgcccttggtgcc
ccttctggat
ccgcagcagc
gccgccgacg

RP2 sh

aggacagatgaccagcttgaagttgaggaggtcgtctggatct
gagaacgatatcacacgtcttgggcaggttcagctcgttatgt

(127) ESL

primer
CW 649 cont. 9

aggttc tatttcaggcagcgctcaaagtaggtgggagce
gatgtagcca cccc gcatgg ag cgctgcacgttc
tgctcaaacag ccg cc gg ttg ttctgc aggacctct
gacggccc tcctttg ttcag tggg tcc tc gggg ttgggctcc
aagaagagatactgcaggccataaatt atggagttatc
gtaaggactggc

cw 54
primer
gcattgg taactgtcagaccaag tttactcatatata
cttagatt gatttaattctcatgtttgacagcttatcaac
gataagcttctattcaggcagcgctcaaagta
ggtgggagccgatgtagcca cccc gcatgg

Exhibit B to Accompany Declaration under 37 CFR 1.131 and Amendment and Response to May 3, 2006 Office Action in USSN 10/681,690

```
 +1  M  I  K  L   F  S  L   K  Q  Q   K  K  E  E   E  S  A
  1  ATGATCAAGC TGTTCTCGCT GAAGCAGCAG AAGAAGGAGG AGGAGTCGGC
     TACTAGTTCG ACAAGAGCGA CTTCGTCGTC TTCTTCCTCC TCCTCAGCCG
-------------------------------------------------------------------------------

 +1    G  G  T   K  G  S  S   K  K  A   S  A  A   Q  L  R
 51  GGGCGGCACC AAGGGCAGCA GCAAGAAGGC GTCGGCGGCG CAGCTGCGGA
     CCCGCCGTGG TTCCCGTCGT CGTTCTTCCG CAGCCGCCGC GTCGACGCCT
-------------------------------------------------------------------------------

 +1  I  Q  K  D   I  N  E   L  N  L  P   K  T  C   D  I  S
101  TCCAGAAGGA CATAAACGAG CTGAACCTGC CCAAGACGTG TGATATCAGC
     AGGTCTTCCT GTATTTGCTC GACTTGGACG GGTTCTGCAC ACTATAGTCG
-------------------------------------------------------------------------------

 +1  F  S  D  P   D  D  L   L  N  F   K  L  V  I   C  P  D
151  TTCTCAGATC CAGACGACCT CCTCAACTTC AAGCTGGTCA TCTGTCCTGA
     AAGAGTCTAG GTCTGCTGGA GGAGTTGAAG TTCGACCAGT AGACAGGACT
-------------------------------------------------------------------------------

 +1    E  G  F   Y  K  S  G   K  F  V   F  S  F   K  V  G
201  TGAGGGCTTC TACAAGAGTG GGAAGTTTGT GTTCAGTTTT AAGGTGGGCC
     ACTCCCGAAG ATGTTCTCAC CCTTCAAACA CAAGTCAAAA TTCCACCCGG
-------------------------------------------------------------------------------

 +1  Q  G  Y  P  H  D  P   P  K  V  K   C  E  T   M  V  Y
251  AGGGTTACCC GCATGATCCC CCCAAGGTGA AGTGTGAGAC AATGGTCTAT
     TCCCAATGGG CGTACTAGGG GGGTTCCACT TCACACTCTG TTACCAGATA
-------------------------------------------------------------------------------

 +1  H  P  N  I   D  L  E   G  N  V   C  L  N  I   L  R  E
301  CACCCCAACA TTGACCTCGA GGGCAACGTC TGCCTCAACA TCCTCAGAGA
     GTGGGGTTGT AACTGGAGCT CCCGTTGCAG ACGGAGTTGT AGGAGTCTCT
-------------------------------------------------------------------------------

 +1    D  W  K   P  V  L  T   I  N  S   I  I  Y   G  L  Q
351  GGACTGGAAG CCAGTCCTTA CGATAAACTC CATAATTTAT GGCCTGCAGT
     CCTGACCTTC GGTCAGGAAT GCTATTTGAG GTATTAAATA CCGGACGTCA
-------------------------------------------------------------------------------

 +1  Y  L  F  L   E  P  N   P  E  D  P   L  N  K   E  A  A
401  ATCTCTTCTT GGAGCCCAAC CCCGAGGACC CACTGAACAA GGAGGCCGCA
     TAGAGAAGAA CCTCGGGTTG GGGCTCCTGG GTGACTTGTT CCTCCGGCGT
-------------------------------------------------------------------------------

 +1  E  V  L  Q   N  N  R   R  L  F   E  Q  N  V   Q  R  S
451  GAGGTCCTGC AGAACAACCG GCGGCTGTTT GAGCAGAACG TGCAGCGCTC
     CTCCAGGACG TCTTGTTGGC CGCCGACAAA CTCGTCTTGC ACGTCGCGAG
-------------------------------------------------------------------------------

 +1    M  R  G   G  Y  I  G   S  T  Y   F  E  R   C  L  K
501  CATGCGGGGT GGCTACATCG GCTCCACCTA CTTTGAGCGC TGCCTGAAAT
     GTACGCCCCA CCGATGTAGC CGAGGTGGAT GAAACTCGCG ACGGACTTTA
-------------------------------------------------------------------------------

 +1  *
551  AG
     TC
-------------------------------------------------------------------------------
```